C4 MMD37

15. (Amended) The method of claim 11, wherein the gene is associated with a cancer, a central nervous system disorder, a blood disorder, a metabolic disorder, a cardiovascular disorder, an autoimmune disorder, or an inflammatory disorder.

C5

24. (Amended) The method of claim 11, wherein the gene is in a cell.

REMARKS

Claims 1-47 are pending in the subject application. Claims 2, 3, 5, 13, 14, 17-23, 29, 34-41 and 47 have been withdrawn from consideration. Claims 27, 28 and 30-33 have been canceled. No claims have been added. Claims 1, 6, 11, 12, 15 and 24 have been amended to make certain format changes. Applicant maintains that these changes raise no issue of new matter, and respectfully request entry of this Amendment. Upon entry of this Amendment, claims 1, 4, 6-12, 15, 16, 24-26 and 42-46 will be pending and under examination.

Pursuant to the requirements of 37 C.F.R. §1.121, applicant annexes hereto as Exhibit A claims 1, 6, 11, 12, 15 and 24 marked up to show the amendments made herein relative to the most recent version thereof.

Invention as claimed

This invention provides a novel chimeric protein that methylates the promoter of a gene, thus inhibiting expression of the gene. This invention also provides a method for inhibiting the expression of the target gene by specifically methylating the promoter sequence with the chimeric protein mentioned above, thus inhibiting

the expression of the gene. By way of example, the inventor demonstrates the successful inhibition of HIV-1 replication in T lymphocytes infected with HIV-1 as well as the successful inhibition of HIV-1 5' LTR-dependent transcription in cultured human HL2/3 cells.

Rejection Under 35 U.S.C. §112, First Paragraph

Written Description

The Examiner rejected claims 1, 4, 6-12, 15, 16, 24-28, 30-33 and 42-46 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Applicant has canceled claims 27, 28 and 30-33 without conceding the correctness of the Examiner's rejection, thereby rendering the rejection of those claims moot.

In response to the rejection of claims 1, 4, 6-12, 15, 16, 24-26, and 42-46, applicants respectfully traverse.

Applicant emphasizes that he is claiming a **chimeric protein** for inhibiting the expression of a gene of interest. Applicant teaches that by methylating the promoter of a gene, expression of that gene can be inhibited. Applicant provides a disclosure which enables the skilled artisan to make and use the chimeric protein combined with what is already known in the art.

Applicant maintains that it is irrelevant whether he discloses a specific example of a chimeric protein which targets a gene

associated with cancer or AIDS because one of skill in the art would know that **all** mammalian promoters tested to date have been found to be silenced when they contain 5-methylcytosine (m^5c) at CpG sites (reviewed by Bestor, 1990; Meehan et al., 1993). Hence, what is important is that the gene promoter of interest, certainly of any mammalian gene, contain a CpG methylation site. If so, that site can be methylated and the expression of that gene can be inhibited.

As of the effective filing date, one of skill in the art would have known how to recognize a methylation site in a promoter sequence. Indeed, applicant's specification teaches that the CpG sequence in promoters is such a putative methylation site (specification at page 8, legend of Figure 12). It would have been routine for one of skill in the art to test whether a target promoter sequence contains a methylation site, and if it does, provided with guidance from applicant's invention, inhibit the expression of that gene.

Additionally, one of skill would also know of many binding proteins for many genes, including those associated with cancer prior to applicant's effective filing date. For example, Ray, et al. report that human breast carcinoma cells transfected with the gene encoding a c-myb promoter-binding protein (MBP-1) inhibits tumors in nude mice (Cancer Res 1995 Sep 1;55(17):3747-51); Chaudhary, et al. report that the c-myc promoter binding protein (MBP-1) and TBP bind simultaneously in the minor groove of the c-myc P2 promoter; and Bloch, et al. report that regulation of c-myb expression in ML-1 human myeloblastic leukemia cells by c-ets-1 protein (Adv. Enzyme Regul. 1995;35:35-41).

The Examiner asserted that specification does not disclose a chimeric peptide which specifically targets a promoter of an

endogenous gene associated with cancer.

As mentioned above, the specification provides all that is needed to construct a chimeric peptide combined with what was already known in the art. It is irrelevant whether the specification specifically discloses the construction of chimeras that methylate CpG sites of promoters associated with cancer genes or HIV. What is important is that the gene of interest contain a CpG site, because, as mentioned above, one of skill would know that all mammalian promoters tested to date have been found to be silenced when then contain 5-methylcytosine (m^5C) at CpG sites.

The Examiner also alleged that the specification does not provide any polynucleotide sequences which encode the chimeric proteins of the elected invention.

As mentioned above, the specification provides enough guidance to create a chimeric peptide which works. Applicant teaches the identification of CpG sites in the HIV-1 5' LTR that yield maximal suppression of transcription when methylated (see Figures 8 and 17). Applicant additionally teaches the selection of zinc finger proteins that bind to predetermined sequences in the HIV-1 5' LTR (Figure 9). Lastly, applicant teaches the successful inhibition of HIV-1 replication in T lymphocytes infected with HIV-1 as well as the successful inhibition of HIV-1 5' LTR-dependent transcription in cultured human HL2/3 cells.

The Examiner alleged that there is no written description of a chimeric protein which comprises a DNA binding sequence that specifically targets the promoter of an endogenous gene which is associated with cancer.

In response, applicants respectfully disagree with the Examiner's

position for the reasons already stated.

In light of the above remarks, applicants maintain that claims 1, 4, 6-12, 15, 16, 24-26 and 42-46 satisfy the requirements of 35 U.S.C. §112, first paragraph.

Rejection Under 35 U.S.C. 112, First Paragraph

Enablement

The Examiner rejected claims 1, 4, 6-12, 15, 16, 24-28, 30-33 and 42-46 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification as to enable one skill in the art to which it pertains, or with which it most nearly connected, to make and/or use the invention.

Applicant has canceled claims 27, 28 and 30-33 without conceding the correctness of the Examiner's rejection, thereby rendering the rejection of those claims moot.

In response to the rejection of claims 1, 4, 6-12, 15, 16, 24-26, and 42-46, applicants respectfully traverse.

Applicant teaches the successful methylation and inhibition of HIV-1 replication in T-lymphocytes infected with HIV-1 as well as the successful inhibition of HIV-1 LTR-dependant transcription in cultured human HL2/3 cells. Applicant teaches the identification of CpG sites in the HIV-1 5' LTR whose methylation produces maximal repression transcription (see Example 2, page 42). Applicant teaches the design, selection and affinity maturation of zinc finger-DNA methyltransferase chimeras that methylate critical CpG sites in the HIV 5' LTR (see Example 3, page 44).

As already mentioned, applicant provides all the guidance that is necessary when combined with what is already known in the art to make and use the claimed invention. Applicant provides a working example of the claimed invention which is effective in silencing transcription of a specific gene of interest.

The Examiner alleged that the specification does not disclose any specific chimeric proteins to be used in the method, nor does the specification disclose any specific polynucleotide which encodes the chimeric protein to be used in the claimed methods.

In response, applicant respectfully disagrees for the reasons set forth above.

The Examiner alleged while it is hypothesized that DNA methylation is involved in numerous processes, including carcinogenesis, the hypotheses remain to be tested.

As already mentioned, all mammalian promoters tested to date have been found to be silenced when they contain 5-methylcytosine at the CpG sites. The Examiner has provided no evidence to support her position that this is not true.

The Examiner alleged that with respect to the elected invention, the specification does not disclose the requisite desired reduction in affinity for an endogenous promoter which is associated with cancer, or what the structural characteristics of such mutants would be.

In response, applicant respectfully disagrees for the reasons set forth above.

The Examiner alleged that the specification does not disclose a

chimeric protein which is capable of binding to a predetermined target promoter sequence, wherein the promoter sequence is endogenous and associated with cancer.

As discussed above, applicant provides all that is necessary to construct a chimeric protein when combined with what is already known in the prior art. As conceded by the Examiner, applicant has previously disclosed two mammalian promoter sequences associated with cancer. Hence, one of skill in the art, provided with guidance from the specification and combined with what is known in the art would be able to construct a chimeric protein which binds close enough to the gene promoter of interest, thereby methylating it and silencing transcription of the gene.

The Examiner alleged that gene therapy is not a routine or predictable art. The Examiner alleged that there is no objective evidence of record that a vector comprising a polynucleotide encoding the non-disclosed chimeric protein can be administered in vivo such that the vector is targeted to the appropriate cell/tissues, is expressed at an appropriate level, and is capable of inhibiting the expression of a specific gene via methylation of a specific endogenous promoter sequence of a gene associated with cancer.

Again, without conceding the correctness of the Examiner's rejection, applicant has canceled claims 27, 28 and 30-33 without prejudice.

In view of the above remarks, applicant maintains that claims 1, 4, 6-12, 15, 16, 24-26 and 42-46 satisfy the requirements of 35 U.S.C. §112, first paragraph, and respectfully request that the Examiner reconsider and withdraw the above-grounds of rejection.

Rejection Under 35 U.S.C. 112, Second Paragraph

The Examiner rejected claims 1, 4, 6-12, 15, 16, 24-28, 30-33 and 42-46 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner alleged that claims 1 and 6 are rendered vague and indefinite by the phrase "attenuated DNA binding activity" as it is unclear as to what the "attenuated DNA binding activity" is. The Examiner suggested adding language such as "relative to said naturally occurring DNA methyltransferase" to overcome this rejection.

Applicant points out that claims 27, 28 and 30-33 have been canceled, rendering the rejection thereof moot.

In response to the rejection of the remaining claims, but without conceding the correctness of the Examiner's position, applicant has amended claim 1 to include the phase "is attenuated relative to that of naturally occurring DNA methyltransferase". Applicants have additionally amended claim 6 to include the phrases "is attenuated relative to that of naturally occurring *M.SssI* DNA methyltransferase" and "is attenuated relative to that of naturally occurring mammalian DNA methyltransferase". Applicant maintains that the amendments to claims 1 and 6 and those claims dependent therefrom overcome the stated rejections.

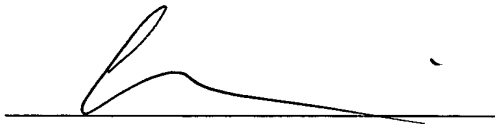
In view of the above remarks, applicant maintains that claims 1, 4, 6-12, 15, 16, 24-26 and 42-46 satisfy the requirements under 35 U.S.C. §112, second paragraph.

In view of the amendments to the claims set forth above, applicant maintains that the Examiner's rejections have been overcome, and respectfully requests that she reconsider and withdraw same.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone at the number provided below.

No fee, other than the $155.00 fee for the two-month extension, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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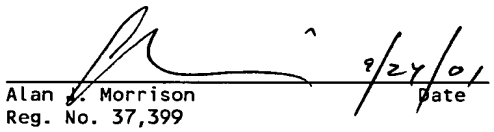

9/24/01
Date

Alan J. Morrison
Reg. No. 37,399

1. A chimeric protein for inhibiting the expression of a gene which comprises (1) a DNA methyltransferase [with attenuated] whose DNA-binding activity is attenuated relative to that of naturally occurring DNA methyltransferase, and [linked to] (2) a DNA binding protein linked thereto that binds sufficiently close to [a] the gene's promoter sequence [of a target gene, which promoter sequence contains a methylation site,] to permit methylation [specifically methylate the] of a methylation site within the promoter, [site and inhibit activity of the promoter and] thus inhibiting [inhibit] expression of the [target] gene.

6. The chimeric protein of claim 1, wherein the DNA methyltransferase [comprises] is a *M.SssI* DNA methyltransferase [protein with attenuated] whose DNA binding activity is attenuated relative to that of naturally occurring *M.SssI* DNA methyltransferase, or a mutated mammalian DNA methyltransferase [protein with attenuated] whose DNA binding activity is attenuated relative to that of naturally occurring mammalian DNA methyltransferase.

11. A method for inhibiting expression of a [target] gene which comprises contacting a promoter of the [target] gene with the chimeric protein of claim 1 so as to [specifically] methylate the promoter, thus inhibiting expression of the [target] gene.

12. The method of claim 11, wherein the [target] gene is an endogenous target gene.

15. The method of claim 11, wherein the [target] gene is associated with a cancer, a central nervous system disorder, a blood disorder, a metabolic disorder, a cardiovascular disorder, an autoimmune disorder, or an inflammatory disorder.

24. The method of claim 11, wherein the [target] gene is in a cell.